OFFICE OF NAVAL RESEARCH

CONTRACT N00014-88-C-0118

TECHNICAL REPORT 92-10

ERYTHROPOIETIN IN RENAL FAILURE: BLOOD RHEOLOGY AND 2,3-DPG
LEVELS

BY

J.P. CROWLEY, J.A. CHAZAN, J.B. METZGER, L. PONO,
AND
C.R. VALERI

NAVAL BLOOD RESEARCH LABORATORY
BOSTON UNIVERSITY SCHOOL OF MEDICINE
615 ALBANY STREET
BOSTON, MA   02118

23 DECEMBER 1992

Reproduction in whole or in part is permitted for any purpose of
the United States Government.

Distribution of this report is unlimited.

ERYTHROPOIETIN IN RENAL FAILURE: BLOOD RHEOLOGY AND 2,3-DPG LEVELS

James P. Crowley, M.D.°
Joseph A. Chazan, M.D.*
Jaclyn B. Metzger, B.S.°
Lucille Pono, R.N.*
C. Robert Valeri, M.D.Δ

From the Department of Medicine, Brown University, and the Divisions of Clinical Hematology° and Renal Disease*, Rhode Island Hospital, Providence, Rhode Island, the Artificial Kidney Center of Rhode Island* and the Naval Blood Research Laboratory, Boston University School of Medicine, Boston, Massachusetts.Δ

The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

Reprint requests can be sent to:

James P. Crowley, M.D.
Division of Clinical Hematology
Rhode Island Hospital
593 Eddy Street
Providence, RI 02903
401-444-4538

ABSTRACT

Twenty-seven transfusion dependent patients with end-stage renal disease on long-term dialysis had blood cell counts, serum chemistries, blood pressure and whole blood viscosity measured, as well as having transfusion requirements assessed. Three months after the institution of rHU-EPO (75 u/kg/wk), there was an 88% fall in transfusion requirement. After 4 months the hematocrit increased from 24 ± 3.8 to $25.6 \pm 4.2\%$, MCV from 93 ± 4.9 to 97 ± 6.6 fl, 2,3-DPG from 13.2 ± 3.2 to 15.6 ± 4.3 µM/gm Hb. Whole blood viscosity fell from 14.1 ± 2.1 to 12.7 ± 2.3 sec. and ferritin levels fell from 3282 ± 3889 to 2131 ± 2441 ng/ml. In 8 patients in whom the dose of rHU-EPO was further increased by up to 50 units/kg three times weekly for 3 months, the hematocrit rose further to $29.3 \pm 3.0\%$ and the rise in hematocrit was accompanied by a further increase in 2,3-DPG to 17.9 ± 2.8 µM/gm Hb ($p<0.03$). There were no major side effects or vascular complications.

♦KEY WORDS: Erythropoietin, transfusion, oxygen delivery, whole blood viscosity, anemia.

2

INTRODUCTION

Previous studies have documented that a substantial population of anemic patients on long term hemodialysis (LTD) are transfusion dependent (4). This anemia can be corrected by the administration of recombinant human erythropoietin, (rHU-EPO) (2,7,20). However, exacerbation of hypertension and an increased tendency to clot vascular access sites have been observed by the Canadian erythropoietin study group (2) in rHU-EPO treated patients.

These workers performed a double-blind, randomized placebo controlled study in 118 patients receiving long-term hemodialysis whose initial hemoglobins were less than 9.0 gm/dl. Patients were randomized to placebo, low dose erythropoietin to achieve a hemoglobin between 9.5 and 11.0 gm/dl or high dose erythropoietin to achieve a hemoglobin between 11.5 and 13.0 gm/dl. Compared with the placebo group, patients treated with erythropoietin had significant improvement in the scores for fatigue, physical symptoms, relationships and depression. However, there was no significant difference in the improvement of quality of life or exercise capacity between the two groups taking the different doses of erythropoietin. Patients taking erythropoietin had a significantly increased diastolic blood pressure despite an increase in either the dose or number of antihypertensive drugs that they were given. Eleven of 78 patients (14%) vs. 1 of 40 given placebo (2.5%) experienced clotting of access sites. Four patients, two in the low erythropoietin group and two in the high erythropoietin group had hypertension that was sufficiently

3

severe for their physicians to withdraw them from the study. No patient was withdrawn from the placebo group.

The mean dose of erythropoietin given in the Canadian low dose erythropoietin study group was approximately 200 units/kg/week. The data presented in the Canadian erythropoietin study showed that the lower erythropoietin dose was equally efficacious and associated with similar side effects. Because of this finding, we wondered whether there would be any beneficial effects of even lower doses of erythropoietin. Although we did not have sufficient numbers of patients to investigate the issue in a double-blind randomized placebo control study, we did have an extensively characterized (4) population of patients who were transfusion dependent. We gave very low doses of rHU-EPO to this group in order to determine whether these minimal doses would be effective in diminishing transfusion requirement. We selected a dose of 75 units/kg/week for our study since previous studies (7) indicated that this dose would have little or no effect on raising pre-transfusion hematocrit.

We were specifically interested in whether red blood cell factors that affect oxygen delivery might be altered by low dose erythropoietin treatment. A recent study by Paulitschke et al. (17) documented an improved red blood cell (RBC) deformability in uremic hemodialysed children by micropipette technique following 30 weeks of treatment with rHU-EPO at doses between 15-100 U/kg/week to maintain hematocrits at approximately 30 vol%. The authors suggested the observed improvement in red blood cell deformability might offset increases in blood viscosity that would occur subsequent to the increase in hematocrit. Moreover, previous studies (18) have

4

suggested that microcytosis increases blood viscosity for any level of hematocrit and macrocytosis has the opposite effect. Relative macrocytosis (an increase in MCV) as a consequence of reticulocytosis is known to occur following rHU-EPO therapy (17). In addition, young red blood cells are known to have relatively higher 2,3-DPG levels than RBC of average age (19) which would be another alteration of RBC characteristics that could enhance oxygen delivery in the microvasculature. Previous studies have emphasized the role of pH in explaining the less than expected levels of 2,3-DPG in dialyzed patients (9,19) rather than the relative paucity of younger red blood cells.

The results to be presented demonstrate that there is a significant decrease in transfusion requirements despite only a modest increase in hematocrit after very low dose rHU-EPO. Moreover, 2,3-DPG levels rose and whole blood viscosity fell in patients treated with rHU-EPO suggesting that improved oxygen delivery may have been a determinant in the decreased transfusion requirement.

METHODS

I. Dialysis Population

Twenty-seven patients, 9 males, 18 females, ages 26-85 receiving hemodialysis at the Artificial Kidney Center of Rhode Island (AKC) were studied over an eleven month period. All of the patients chosen were anemic (hemoglobin 8.1 ± 1.3) and transfusion dependent (mean number of units of blood received in the previous six months=9 ± 6). Two patients died during the study period. The cause of death for one was cancer and for the other was coronary artery disease.

II. Erythropoietin Therapy

All of the patients received 25 U/kg body weight of recombinant human erythropoietin (rHU-EPO, Ortho Pharmaceuticals) three times weekly for eight months. At the end of the 8 month study period the hematocrits of a subset of 8 patients were not greater than 30%. Each of these eight patients were then given a larger dose of 75 u/kg body weight three times weekly (225 u/kg/week) for an additional three months.

III. Blood Samples

Blood for analysis was collected immediately prior to the administration of the first dose of erythropoietin and monthly thereafter. Blood for analysis was obtained by venipuncture from the patients prior to the administration of heparin, blood products, rHU-EPO which were given intravenously prior to hemodialysis treatment.

Blood samples collected on the evening shift were refrigerated overnight and then transported to the laboratory for testing.

IV.   Hematologic Measurements

Complete blood counts including a platelet count were performed on EDTA-anticoagulated blood using a Coulter Counter T540(Coulter Electronics, Hialeah, FL). Red blood cell indices were calculated manually from the hemoglobin,hematocrit and red blood cell count using the standard formulae. Fibrinogen was measured by a thrombin time method using an automated Coag-a-Mate X2(Organon Teknica, Durham, NC). Samples of reticulocyte-rich and reticulocyte-poor blood were prepared by centrifuging 5 ml of EDTA-anticoagulated blood for 1 hour at 3000g. After removing the plasma and buffy coat, the top 0.5 mL of RBCs was removed to a clean tube labelled reticulocyte-rich RBCs. A clean pipette was then placed at the bottom of the centrifuged tube and the bottom 0.5 mL of RBCs was removed to a second clean tube labelled reticulocyte-poor RBCs. Reticulocytes were enumerated using a standard New Methylene Blue dye technique and the number of reticulocytes seen in 1000 RBCs was expressed as a percent. The absolute reticulocyte count was calculated by multiplying the percent of reticulocytes by the RBC count of the corresponding sample. The absolute reticulocyte count in the reticulocyte-rich fraction was significantly higher than in the reticulocyte-poor fraction $(8.6 \times 10^6/\text{ml}$ vs $2.1 \times 10^6/\text{ml}$, $p < 0.001$, N=8).

V.   Viscosity Measurements

Whole blood viscosity was measured using a porous bed viscometer (Merrill Medical Corporation, Belmont, MA)[5]. Each

blood sample was warmed for 30 minutes at 37°C. before being measured in a 37°C. viscometer.

VI. Biochemical Measurements

Ferritin was measured by RIA. Urea nitrogen and creatinine were measured by a standard kinetic method. Electrolytes were measured using an ion selective electrode. Total protein and albumin were measured by a standard colorimetric technique. RBC 2,3-DPG was measured spectrophotometrically using a commercial kit (Sigma Diagnostics, St. Louis, MO).

VII. Statistical Analysis

The mean and standard deviation (mean±SD) were reported for each parameter measured at each time point. Standard error of the mean (SEM) was noted where reported on a figure. A repeated measures analysis of variance (ANOVA) was carried out for each parameter over the study period. Some parameters were routinely measured monthly by the dialysis center and thus were analyzed for longer periods to confirm trends. Trends were confirmed in each case of a significant ANOVA by a correlation test. The total number of patients (N) studied was 27. However, due to hospitalizations or death, some patients were not studied at every time point. Thus, the N reported for each ANOVA may vary (e.g. N-23), but the correct N is reported for each figure. A p value of <0.05 was considered significant for all tests.

RESULTS

There was a small but statistically significant ($p<0.02$) increase in hematocrit in the transfusion dependent patients treated with very low dose erythropoietin (Table 1, 24 ± 3.8 vs. 25.6 ± 4.2) at four months. The increase in hematocrit persisted throughout the 8 months of study (Figure 1). There were no significant changes in fibrinogen levels, BUN, albumin, sodium or potassium levels. There was no significant rise in either the mean or the systolic or diastolic blood pressure (Table 1). Ferritin levels dropped about one-third of pre-rHU-EPO treatment values. Transfusion requirements fell dramatically in patients treated with rHU-EPO, an 88% fall being evident 8 months following initiation of rHU-EPO therapy (Figure 2). Whole blood viscosity levels fell from 14.1 ± 2.1 to 12.7 ± 2.3 ($p<0.03$, Table 1) and an increased MCV was evident over baseline throughout the additional 4 months of study (Figure 1). MCV rose from $93. \pm 4.9$ to 97 ± 6.6 over the same time period.

RBC 2,3-DPG levels rose from 13.2 ± 3.2 to 15.6 ± 4.3 ($p<0.02$, Table 1), and these levels of greater than 20% of baseline values persisted throughout the 8 months of the study (Figure 1). Further increases in both hematocrit and RBC 2,3-DPG levels could be obtained when patients treated initially for 8 months with very low doses of rHU-EPO were treated with higher doses of 225 units/kg/weekly (Figure 3).

RBC 2,3-DPG levels in reticulocyte rich and reticulocyte poor fractions of whole blood were studied in 8 patients after treatment with rHU-EPO for 8 months. The 2,3-DPG levels in these patients

9

before and after rHU-EPO treatment for 8 months were 12.1 ± 3.1 and $16.0 \pm 3.7 \mu m/gmHb$ respectively (p<0.01). These studies demonstrated that the increased RBC 2,3-DPG levels were more pronounced in the reticulocyte rich fraction ($17 \pm 2.8 \mu m/gmHb$) than the reticulocyte poor fraction ($14.7 \pm 3.9 \mu m/gmHb$), but the difference was not significant. Increased RBC 2,3-DPG was also present in the reticulocyte poor fraction, indicating that the effect of rHU-EPO on increasing RBC 2,3-DPG levels persisted in older red blood cells despite similar dialysis conditions throughout the period of the study.

DISCUSSION

Although the improvement in hematocrit was only modest (24.5 ± 3.8 to 25.6 ± 4%, N=27), within three months of therapy there was an 88% decrease in the number of red blood cell units transfused/patient/month (Figure 2). This finding is consistent with several previous studies that have demonstrated a reduction or elimination of transfusion requirements and improvement in hematocrit following therapy with recombinant human erythropoietin at higher doses (2,6,7,20). This study differs from previous studies in using a very low dose (25 units/kg I.V. three times a week) for several months. Since a previous study in the same population (4) showed that most transfusions are self initiated by complaints of fatigue, it is reasonable to conclude that fatigue was reduced in these patients. Since the present study was not placebo controlled or double-blinded, the possibility that the patients or physicians may have been influenced by the knowledge that they were receiving rHU-EPO cannot be ruled out although it seems unlikely. The results we observed would strongly support a larger placebo controlled, double-blinded study of very low doses of rHU-EPO along the lines of the previous study using higher doses performed in Canada (2).

Two blood parameters were observed to change in a way that might enhance oxygen delivery, and thus reduce fatigue: a decrease in whole blood viscosity and an increase in RBC 2,3-DPG levels. Previous studies of whole blood viscosity in patients treated with erythropoietin have demonstrated an increase rather than decrease in whole blood viscosity (1,8). However, the mean hematocrit of these patients rose

11

from 19.4 to 31.7 during the period of the first study and from 29.2 to 39.3 in the second cited study (1). The greater increase in hematocrit would explain the overall increase in whole blood viscosity since hematocrit is the major determinant of whole blood viscosity (5). However, our studies are consistent with the study of Paulitschke et al. (17) who found a decrease in red blood cell deformability and increased red blood cell mean volume following rHU-EPO therapy. Further studies of whole blood viscosity in patients on dialysis will be needed to resolve whether the fall in whole blood viscosity was related to any clinically significant improvement in tissue perfusion.

Correction of the effects of anemia by rHU-EPO on blood pressure are complex (3,11,14,15), but the observed reduction of whole blood viscosity may have also contributed to a stabilization of the mean arterial pressure (Table 1) in the patients studied. Anemia results in vasodilation which decreases peripheral resistance (13). When the anemia is corrected, peripheral resistance will predictably increase which in turn may increase diastolic blood pressure. On the other hand, cardiac output falls as oxygen delivery improves and an overall decrease in mean arterial pressure may be the result (16). Casati et al. (3) in a relatively early report of 14 patients treated with rHU-EPO found that the correction of anemia in 8 previously hypertensive patients exacerbated hypertension, but it was controllable with changes in medications. The rise in blood pressure during erythropoietin treatment was attributed to the increased blood viscosity and total red cell mass inducing an increase in peripheral resistance (3) notable after the attainment of hematocrits >35. In the Canadian erythropoietin study group patients taking low dose

12

erythropoietin (2) also had a significantly increased diastolic blood pressure when hemoglobin concentrations were kept at 9.5 to 11.0 gm/dl (28.5 - 33.0 hematocrit). Our study results show very low dose erythropoietin therapy had only a slight effect on hemoglobin level and rHU-EPO treatment was not associated with significant changes in either systolic or diastolic blood pressure (Table 1).

An important biochemical alteration observed in patients treated with low dose erythropoietin was an increase in RBC 2,3-DPG levels (Table 1, Figure 1). RBC 2,3-DPG levels are raised in anemic patients (19), but it is known that the increase in RBC 2,3-DPG is less than expected in patients on long term hemodialysis (9,19). Previously, the explanation for this was the metabolic affects of acidosis (9). The results of the present study would tend to place more emphasis on mean RBC age since presumably all metabolic factors remain the same after treatment with rHU-EPO and yet RBC 2,3-DPG levels increased. The rise in RBC 2,3-DPG levels was large and was comparable to patients being taken to high altitude (19). Since maximum intracellular adaptation to anemia can produce an improvement in oxygen delivery of up to 30% (19), oxygen delivery would be clearly enhanced by the increases in RBC 2,3-DPG observed after rHU-EPO. It would be of interest to assess the individual and collective contributions of increased RBC 2,3-DPG levels, hematocrit and improved WBV on improved oxygen delivery. Although this would be difficult to study in man, suitable animal models have been developed for looking at oxygen delivery (10,12) which could be used to study further the relative contributions of hematocrit, WBV and 2,3-DPG levels to enhanced oxygen delivery in anemic patients treated with

13

very low doses of rHU-EPO.

REFERENCES

1. Brown CD, Kieran M, Thomas LL: Treatment of azotemic, nonoliguric, anemic patients with human recombinant erythropoietin raises whole blood viscosity proportional to hematocrit. Nephron, 59:394-398, 1991.

2. Canadian Erythropoietin Study Group. Br Med J., 300:573-, . 1990.

3. Casati S, Passerini P, Rosaria C, et al: Benefits and risks of protracted treatment with human recombinant erythropoietin in patients having haemodialysis. Br Med J., 295:1017-1020, 1987.

4. Crowley JP, Nealey TA, Metzger JB, Pono L, Chazan JA: Transfusion and long-term hemodialysis. Arch Intern Med., 147:1925-1928, 1987.

5. Crowley JP, Metzger JB, Gray A, Merrill E, Valeri CR: The disposable porous bed viscometer: A new method for measuring blood viscosity. Amer J Clin Path., 96:729-737, 1991.

6. Erslev AJ: Erythropoietin. New Engl J Med., 324(19):1339-1344, 1991.

7. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW: Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. New Engl J Med., 316:73-78, 1987.

8. Macdougall IC, Davies ME, Hutton RD, et al: Rheological studies during treatment of renal anemia with recombinant human erythropoietin. Br J Haem., 77:550-558, 1991.

9.   Miller ME: Oxygen transport in uremia. Sem in Nephr.,
      5(2):140-141, 1985.

10.   Most AS, Ruocco NA, Gewirtz H: Effect of a reduction in blood
      viscosity on maximal myocardial oxygen delivery distal to a
      moderate coronary stenosis. Circulation, 74(5):1085-1092,
      1986.

11.   Murray JF, Gold P, Johnston BL: The circulatory effects of
      hematocrit variations in normovolemic and hypervolemic dogs.
      J. Clin Invest., 42:1150-1159, 1963.

12.   Murray JF, Escobar E: Circulatory effects of blood viscosity:
      comparison of methemoglobinemia and anemia. J App Phys.,
      25(5):594-599, 1968.

13.   Neff MS, Kim KE, Persoff M, Onesti G, Swartz C: Hemodynamics
      of uremic anemia. Circulation, 43:876-883, 1971.

14.   Nissenson AR, Nimer SD, Wolcott DL: Recombinant human
      erythropoietin and renal anemia: Molecular biology, clinical
      efficacy, and nervous system effects. Ann Intern Med., 114:402-
      416, 1991.

15.   Pascual J, Teruel JL, Ortuno J: Hypertensive effect of
      erythropoietin. Ann Intern Med., 114(12):1063, 1991.

16.   Pascual J, Teruel JL, Liano F, Ortuno J: Decreased blood
      pressure after a year of erythropoietin. Nephron, 58:374-375,
      1991.

17.   Paulitschke M, Meier W, Lerche D, Zoellner K, Schmidt G:
      Positive effect of recombinant human erythropoietin on static
      and dynamic properties of individual red blood cells in uremic
      hemodialysed children. Presented at Sixth European

Conference on Clinical Hemorheology, Frankfurt am Main, Germany, 15-17 June 1989. <u>Clinical Hemorheology</u>, 10:653-664, 1990.

18. Tillman W, Schroter W: Rheological properties of erythrocytes in heterozygous and homozygous B-thalassemia. <u>Br J Haem</u>., 43:401-411, 1979.

19. Valeri CR, Zaroulis CG, Fortier NL: Peripheral red cells as a functional biopsy to determine tissue oxygen tension. In: *Oxygen Affinity of Hemoglobin and Red Cell Acid Base Status*, ed. M. Rorth, P. Astrup, Academic Press, New York, 1971.

20. Winearls CG, Pippard MJ, Downing MR, Oliver DO, Cotes PM: Effect of human erythropoietin derived from recombinant DNA on the anaemia of patients maintained by chronic haemodialysis. <u>Lancet</u>, 8517, 1175-1178, 1986.

ACKNOWLEDGEMENTS

Figure 1:   Relative changes in mean corpuscular volume (MCV), hematocrit and 2,3-DPG levels in a group of 27 long-term dialysis patients treated with 25 units/kg of rHU-EPO three times weekly for eight months.
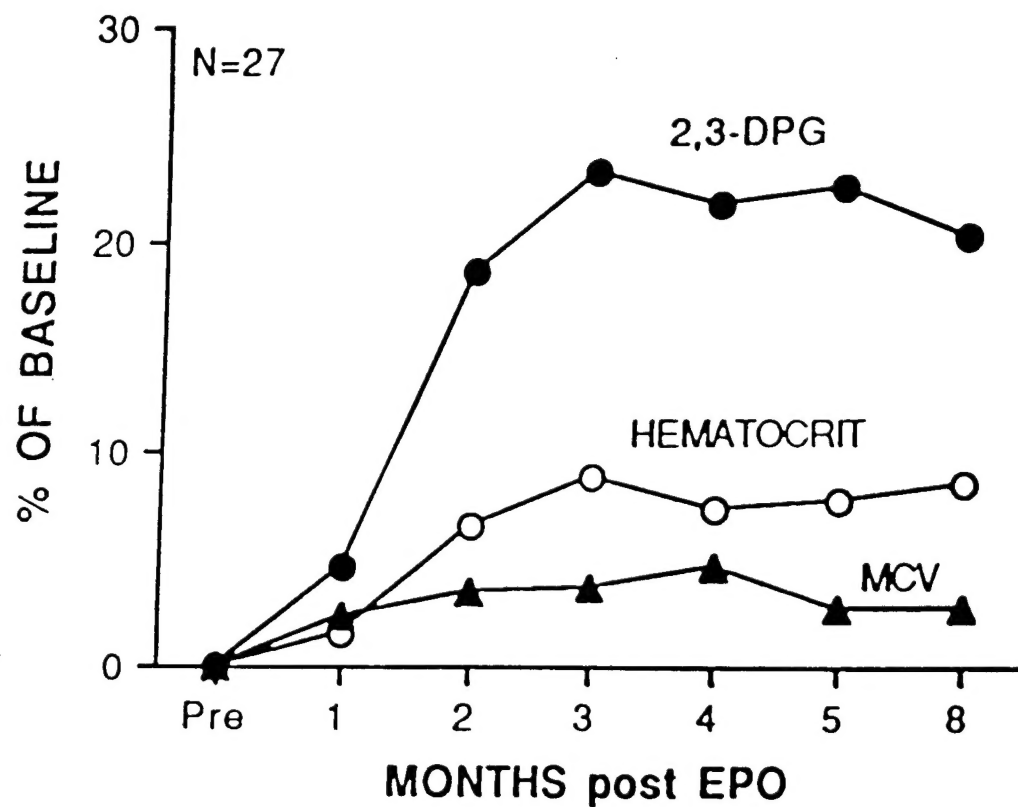
Figure 2:   The effect of treatment with rHU-EPO on the transfusion
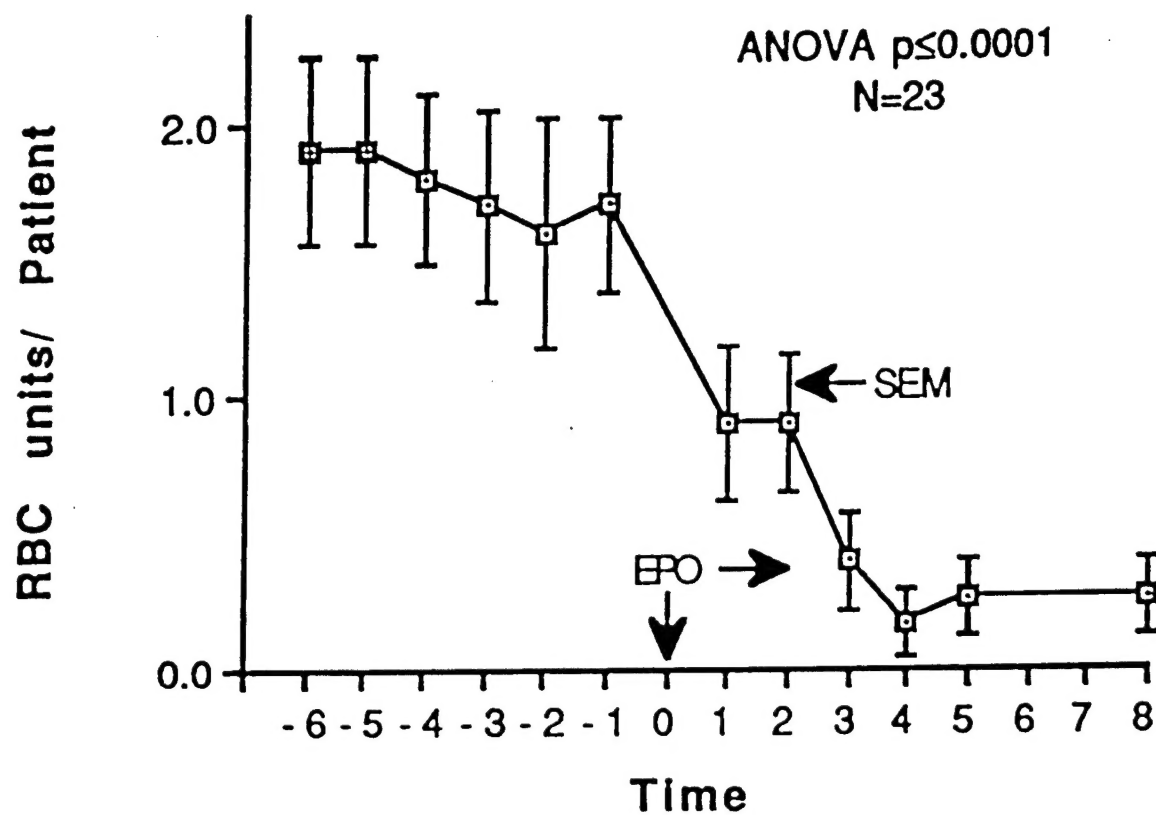requirements of 23 long-term dialysis patients.

Figure 3: Increases in hematocrit and 2,3-DPG levels (mean ± SD) in eight long-term dialysis patients treated for 8 months with rHU-EPO at a dose of 75 units/kg weekly intravenously then for 3 months at a dose of 225 units/kg weekly.
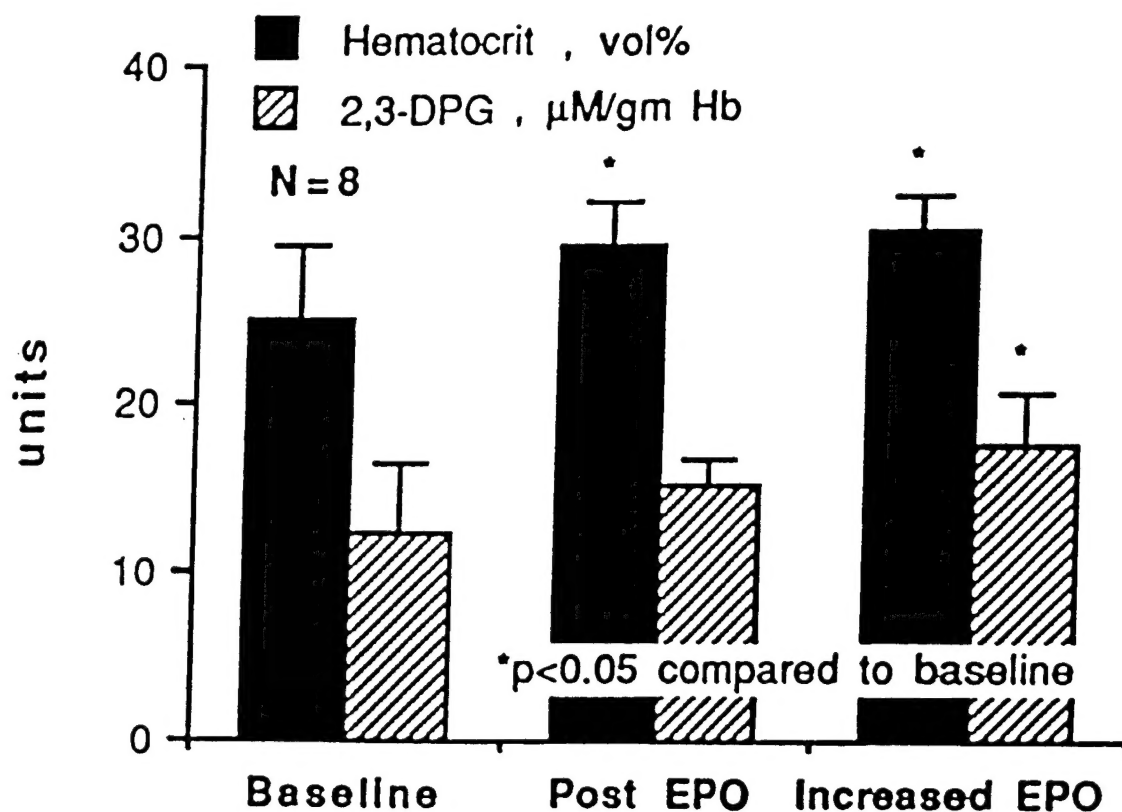
TABLE 1

Changes in blood count, chemistries and arterial pressure observed in 23 transfusion dependent long-term dialysis patients treated with low dose (25 U/Kg) rHU-EPO 3 times weekly for 4 months. The analysis of variance (ANOVA) indicates the significant changes from the 4 months post-EPO.

PARAMETER	Pre-EPO mean±SD	post-EPO (4 mos) mean±SD	ANOVA p≤
WBC x 10^6/mL	6.7±1.8	6.4±1.8	NS
Hemoglobin, gm/dL	8.1±1.3	8.4±1.5	NS
Hematocrit, vol%	24±3.8	25.6±4.2	0.02
MCV, fl	93±4.9	97±6.6	0.001
Platelet x 10^6/mL	184.5±58.7	203±66	NS
Whole Blood Viscosity, sec	14.1±2.1	12.7±2.3	0.03
2,3-DPG, µM/gm Hb	13.2±3.2	15.6±4.3	0.02
Fibrinogen, mg/dL	387±128	386±110	NS
Ferritin, ng/mL	3282±3889	2131±2441	0.005
BUN, mg/dL	98.3±22	101.4±26	NS
Sodium, mEq/L	136±6	138±4	NS
Potassium, mEq/L	5±0.9	5.1±0.8	NS
Total protein, gm/dL	6.8±0.6	6.7±0.7	NS
Albumin, gm/dL	3.8±0.4	3.7±0.3	NS
Mean arterial pressure, mmHg	93±11	95±10	NS
Systolic arterial pressure	138±21	142±18	NS
Diastolic arterial pressure	70±10	71±10	NS